Food Microbiology 20 (2003) 549–559

FOOD MICROBIOLOGY

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Estimation of growth of *Clostridium perfringens* in cooked beef under fluctuating temperature conditions

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Received 6 September 2002; accepted 4 November 2002

Abstract

A new concept for estimating the bacterial growth under temperature fluctuations was hypothesized and validated using *Clostridium perfringens* as a test organism. This new methodology was based on the Gompertz models to calculate the equivalent growth times under different temperatures, and estimated the bacterial population under temperature fluctuations. The new concept was tested in ground beef maintained under fluctuating temperature conditions. The estimation accuracy of this methodology was generally within 1.0 $\log_{10}$ (cfu/g). Although the methodology was based on *C. perfringens*, it can potentially be applied to other foodborne pathogens to predict the bacterial growth under temperature fluctuations.
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Keywords: Estimation; Microbial growth; *Clostridium perfringens*; Fluctuating temperature

1. Introduction

Clostridium perfringens is a serious foodborne pathogen in the United States, and is a major concern for the food industry (CDC, 1994, 1996; Stringer et al., 1980; Todd et al., 1997). An anaerobic, Gram-positive, spore-forming bacillus, this organism is ubiquitously distributed in the environment and can be readily isolated from dust, raw meat and poultry, and the intestinal tract of man and animals (Granum, 1990). Symptoms of *C. perfringens* gastroenteritis, including acute abdominal pain and diarrhea, usually appear 8–16 h after ingestion of the vegetative form of the organism and can last 24–48 h (Duncan et al., 1972; FDA-CFSAN, 2001; Hall and Angelotti, 1965).

Red meats and poultry products are frequently contaminated with *C. perfringens* (Hall and Angelotti, 1965; Bean and Griffin, 1990; Gross et al., 1989; Shandera et al., 1983; Steele and Wright, 2001). The spores of this organism are more heat-resistant than most foodborne pathogens such as *Salmonella* and *Listeria*. They can survive normal cooking or thermal processing conditions in manufacturing products such as ham, roast beef and corned beef (Craven, 1980). Anaerobic conditions created as a result of oxygen depletion during cooking may enable the spores of this organism in cooked meat and poultry products to germinate, outgrow, and multiply to dangerously high dose levels if product temperatures are not properly maintained (Barnes e al., 1963; Gross et al., 1989).

C. perfringens can grow at temperatures between 6°C and 52°C (FDA, 1998; Hall and Angelotti, 1965; Johnson, 1990; Shoemaker and Pierson, 1976), and can multiply rapidly (generation times between 7 and 30 min) if maintained between 30°C and 47°C (Craven, 1980; Juneja et al., 1999). Rapid cooling is the first critical step to prevent the germination and growth of this organism. The US Department of Agriculture (USDA) requires that the internal temperature of the slowest cooling point in cooked beef, roast beef and cooked corned beef should be cooled from 48.9°C to 12.8°C in 6 h or less, and the cooling continue to 4.4°C prior to boxing (USDA, 1993). The time/temperature guidelines issued by USDA Food Safety Inspection Service (USDA, 1989) recommend that the maximum internal temperature of cooked products should neither

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0740-0020/03/$ - see front matter Published by Elsevier Science Ltd.
PII: S0740-0020(02)00155-7

be maintained between 54.4°C and 26.7°C for more than 1.5 h nor between 26.7°C and 4.4°C for more than 5 h. The Food Code published by the US Food and Drug Administration (FDA) also suggests that all foods should be cooled from 60°C to 21°C in 2 h and from 21°C to 5°C in 4 h (FDA, 2001).

In addition to cooling, *C. perfringens* may grow as a result of inevitable temperature abuses at various stages of distribution, storage, retail and consumption. An accurate estimation of growth of this organism in the products during distribution, storage and retail operations based on the time–temperature histories may help food processors, distributors and retailers adopt proper precautionary procedures to restrict its growth.

Several methods have been proposed to estimate bacterial growth under fluctuating temperature conditions. Li and Torres (1993) proposed a method based on the logistic growth model to estimate the completion of the lag phase and the growth of bacteria in the exponential phase based on the three-phase linear growth model. Based on a modified Gompertz model, Zwietering et al. (1994) hypothesized that the temperature shift within a lag phase would induce an additional lag for the bacterial cells and proposed another method to estimate the bacterial growth with a shift in temperature. They concluded that their method was more accurate than the linear model. Also derived from the Gompertz model, Van Impe et al. (1992, 1995) developed a method using the implicit differential Gompertz equation to dynamically simulate the bacterial growth. Although this method is capable of estimating the growth of micro-organisms under complicated temperature conditions, it involves solving a differential Gompertz equation and requires complex numerical techniques and therefore would be difficult to use for ordinary food processors and retailers who may just want to know how bacteria grow when a food product is brought from one temperature condition to another. The temperature profile of such a scenario can be easily represented by a step function or square-waved functions.

The main objective of this study was to develop an accurate, easy-to-use mathematical method to estimate the growth of *C. perfringens* in cooked ground beef under fluctuating temperature conditions and to compare this method with the methods developed by Li and Torres (1993) and Zwietering et al. (1994). This method was based on the conventional Gompertz growth model and should have an accuracy of approximately $\pm 1.0 \log_{10}$ (cfu/g). Such a methodology would enable different sectors, such as manufacturing, distribution, retail and food service, of the food industry to estimate the potential growth of *C. perfringens* in cooked beef products under simple fluctuating temperature conditions and prevent the unsafe products from entering the market.

2. Concept of equivalent growth time (EGT)

2.1. Isothermal bacterial growth

In the evaluation of the microbial safety of cooked meat products, food microbiologists frequently categorize a bacterial growth process into four general phases—lag, exponential, stationary and decline. The decline phase following the stationary phase, however, is usually ignored since the food under evaluation is microbiologically beyond the limit for consumption after the stationary phase, particularly for *C. perfringens*. Therefore, from the food safety standpoint, the microbial growth is generally described by a sigmoidal function, such as the Gompertz function (Gibson et al., 1987), and mathematically it can be expressed as

$$L(t) = A + (B - A)\exp\{-\exp[-\mu(t - M)]\}, \quad (1)$$

where $L(t)$ is the $\log_{10}$ count of the number of pathogens at time t, $\log_{10}$ (cfu/g); A the initial cell concentration or the asymptotic $\log_{10}$ count as time approaches zero, $\log_{10}$ (cfu/g); B the maximum cell concentration or the asymptotic $\log_{10}$ count as time approaches infinity, $\log_{10}$ (cfu/g); M the time at which the specific growth rate is maximum, h; and μ the relative growth rate at time M, h^{-1}.

Although the Gompertz model is merely an empirical model, it has been widely used and tested in predictive microbiology (Gibson et al., 1987; Juneja et al., 1999; McClure et al., 1994; Zwietering et al., 1990). It may lack biological basis for the growth of micro-organisms, the Gompertz model can accurately describe the bacterial growth from the lag through exponential to stationary phases (McDonald and Sun, 1999). This model is probably the most frequently used growth model in predictive food microbiology. Mathematically, this model is relatively easy to obtain from the growth data and therefore is chosen for describing isothermal bacterial growth in this study.

2.2. Hypothesis of EGT under different temperatures

The Gompertz model (Eq. (1)) developed under an isothermal condition cannot be directly used to calculate the growth of bacteria when there is a change in growth temperature. To use the Gompertz equation in the estimation of bacterial growth under non-isothermal conditions, this paper proposes a hypothesis of EGT or equivalent growth time, for describing and comparing the bacterial growth under different temperature environments.

For the spores of *C. perfringens* mixed in ground beef in a closed system such as a sealed plastic bag, it is assumed that the bacteria are chemically and nutritionally isolated from environmental influences. Ground beef can provide sufficient nutritional requirements to

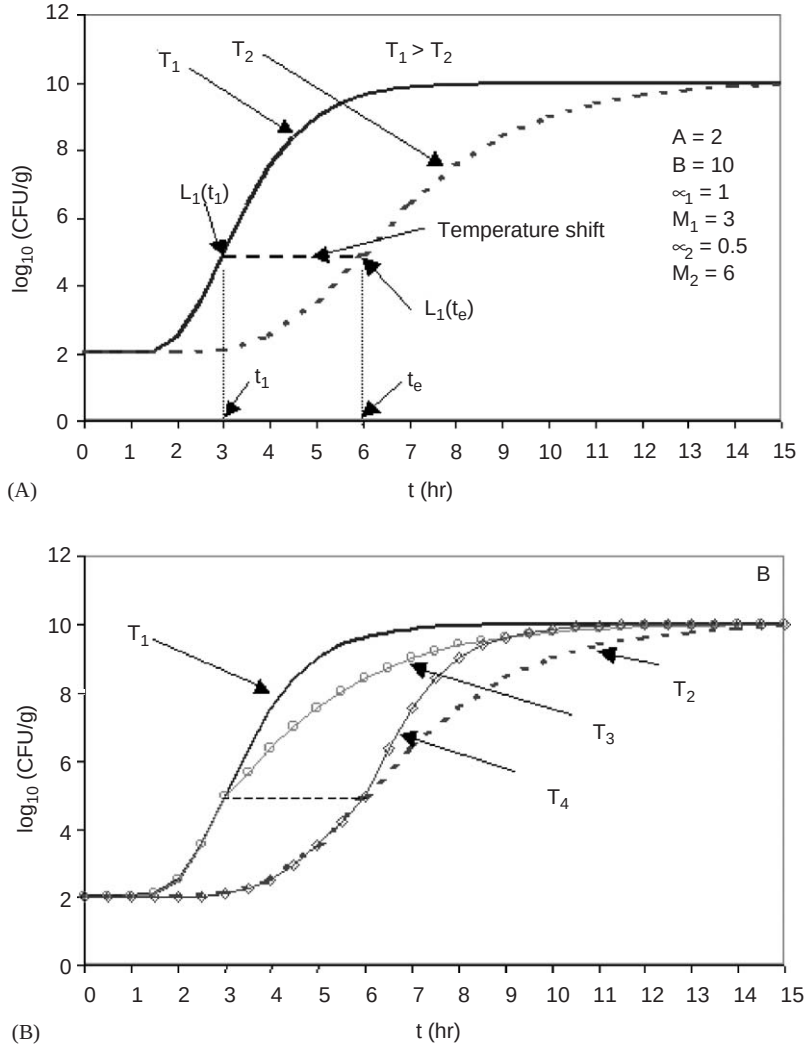

Fig. 1. The concept of EGT. (A) The calculation of EGT between two temperature conditions (T_1 and T_2). (B) The growth curves of two simulated temperature profiles T_3 and T_4.

support the growth of *C. perfringens* within the sealed plastic bag.

Within the limits of the minimum and maximum temperatures, it is assumed that the growth and multiplication of *C. perfringens* cells after the germination of spores in cooked ground beef in a closed system can be considered as a continuous process under isothermal or non-isothermal conditions since nothing can stop the bacterial growth. The bacterial cells would stop growing when the environmental temperature is outside the limits of the minimum and maximum temperatures. The injury to the vegetative cells caused by heat inactivation or low temperature may lead to a decrease in the cell population at temperatures above the maximum or below the minimum growth temperature. A lag phase will be induced as the cells previously exposed to the temperatures outside the growth temperature range are reintroduced to a permissible temperature environment.

Illustrated in Fig. 1A are two isothermal growth curves at T_1 and T_2 ($T_1 > T_2$). The initial and maximum cell concentrations for both curves are 2 and 10 $\log_{10}$ (cfu/g), respectively. It is assumed that a sample is initially incubated at temperature T_1 for any given period of time of t_1, and then at temperature T_2 for another period of time. The bacterial growth in the first incubation period can be calculated from the Gompertz model (Eq. (2))

$$L_1(t) = A + (B - A)\exp\{-\exp[-\mu_1(t_1 - M_1)]\}. \quad (2)$$

After the temperature is shifted, bacterial cells will continue to grow and multiply from the end point of the first incubation period. The initial cell concentration for the second incubation period should be $L_1(t)$. To calculate the bacterial growth after the temperature change, it is assumed that the growth at temperature T_1 for the incubation time of t_1 is equivalent to a growth process at another temperature condition (T_2) for an

incubation time of t_e, mathematically expressed as

$$L_1(t) = A + (B - A)\exp\{-\exp[-\mu_2(t_e - M_2)]\}. \quad (3)$$

In Eq. (3), t_e is the EGT, and is the basis for the calculation of bacterial growth under fluctuating temperature conditions. Based on this concept, the bacterial growth under T_2 for a period of Δt after the temperature shifting can be determined from

$$L_2(t_1 + \Delta t) = A + (B - A) \times \exp\{-\exp[-\mu_2(t_e + \Delta t - M_2)]\}. \quad (4)$$

The EGT (t_e) of any two isothermal conditions can be calculated at any growth stages (lag, exponential and stationary) of micro-organisms. Illustrated in Fig. 1B is the calculation and conversion of the EGTs between two example growth curves (T_3 and T_4). Curve T_3 represents an incubation process that starts at a higher incubation temperature (T_1) for 3 h prior to shifting to a lower incubation temperature (T_2). Curve T_4 is a growth curve that starts at a lower temperature (T_2) for 6 h before the incubation temperature is changed to a higher temperature T_1. Growing at a higher temperature (T_1) for 3 h is equivalent to 6 h growth at the low temperature (T_2) and vice versa. This new concept will take the advantage of many bacterial growth data described by various Gompertz equations, and is particularly suitable for evaluating the bacterial growth under simple square-waved fluctuating temperature profiles frequently encountered in food processing and retailing environments.

3. Materials and methods

3.1. Test organisms and sample inoculation

Three strains of *C. perfringens* spores, NCTC 8238 (Hobbs serotype 2), NCTC 8239 (Hobbs serotype 3), and NCTC 10288 (Hobbs serotype 13), were selected in this study. These strains were obtained from Dr. John Novak of USDA-ARS-ERRC located in Wyndmoor, PA. Spores of *C. perfringens* were prepared using the procedures developed by Juneja et al. (1993). Each spore crop was washed twice, resuspended in sterile de-ionized water and maintained at 4°C until ready for use.

Ground beef (93% lean) purchased from a local grocery store was irradiated to sterility by ionizing γ irradiation at –30°C to a final dose level of 42 KGY using a ^{137}Cs source available in the irradiation facility of USDA-ARS-ERRC located at Wyndmoor, Pennsylvania (Thayer et al., 1995). Aliquots of three spore strains with the same optical density were mixed into the sterilized ground beef (≈1500 g). A Kitchen Aid Mixer was used to mix the bacteria and ground beef for 60 min under refrigerated conditions and a homogeneous distribution of the spores was experimentally confirmed. The inoculated ground beef was divided into 5.00 ± 0.02 g portions and packaged into plastic filter bags (Model BagPage® BP 100, Interscience Co., France). Each plastic bag was vacuum sealed to a final vacuum of 15 mmHg. Samples were kept frozen (−20°C) until use.

3.2. Growth study

The frozen ground beef samples were thawed overnight in a refrigerator (4°C) followed by heat shock at 75°C in a water bath for 20 min to activate the spores and to inactivate any contaminating vegetative cells. After briefly rinsing with running water (≈20°C for 1–2 min) to cool the meat in the plastic bags, samples were incubated isothermally at 30°C, 36°C and 45°C to obtain isothermal growth curves. Samples from each incubation temperature were periodically removed for determination of bacterial cell concentrations. At least three independent experiments were conducted to generate an isothermal growth curve. Although the experiments could be conducted at any temperature within the limits of the minimum and maximum temperatures for *C. perfringens*, this organism could grow at relatively fast rates at the temperatures selected in this study.

To investigate the effect of temperature fluctuations on the growth of *C. perfringens* in cooked ground beef, samples were alternated between two incubators maintained at two temperature ranges (30–45°C or 45–36°C) to simulate fluctuating temperature conditions. At each transfer between two incubators, samples were retrieved to determine cell concentrations.

3.3. Determination of C. perfringens cell concentrations

Samples retrieved from incubators were immediately diluted with equal volumes (5 ml) of 0.1% sterile peptone-water. A rubber hammer was used to gently break the heat-denatured ground beef into small pieces. After that, samples were mixed in a MiniMix Stomacher (Model BagMix® 100 W, Interscience Co.) at maximum speed for 12 min to ensure a complete homogenization of the meat samples. After homogenization, a small volume (0.1–0.5 ml) of the liquid portions was serially diluted with 0.1% sterile peptone-water and plated on Shahidi–Ferguson Perfringens (SFP) agar. After plating, each SFP agar plate was overlaid with approximately 10 ml of freshly prepared SFP agar. After the SFP agar layer was solidified, the plates were placed in an anaerobic chamber (Model Bactron IV, Sheldon Manufacturing Inc., Cornelius, OR) and incubated at 37°C for 24–48 h under an atmosphere of $CO_2/N_2/H_2$ (85%:10%:5%). Typical bacterial colonies were counted and recorded after incubation.

3.4. Analysis of isothermal growth curves

Plate counts of *C. perfringens* were transformed to $\log_{10}$ values and analysed by nonlinear regression to fit the growth data to the Gompertz model (Eq. (1)). Due to the homogeneity of the spores in the ground beef samples, all the experimental growth data of independent experiments under the same growth temperature were combined to generate a general Gompertz model for that temperature. Three Gompertz models for each isothermal condition (30°C, 37°C and 45°C, respectively) were independently generated in this study.

A Windows-based statistical analysis package—NCSS-2000 was used to analyse and fit to the growth data to the Gompertz model (Hintze, 1999). The Levenberg–Marquardt nonlinear least-squares algorithm (Nash, 1987) was used to estimate the parameters of the Gompertz models in the nonlinear regression procedure provided by NCSS–2000. For the nonlinear regression, a pseudo-R^2 value was calculated for each growth curve to approximate the usual R^2 used in linear regression. Although the pesudo-R^2 might not be a perfect indicator for evaluating the goodness of fit for nonlinear regression, it served well for comparative purposes.

3.5. Estimation of bacterial growth under temperature fluctuations

To estimate the growth of *C. perfringens* in cooked ground beef under fluctuating temperature conditions, the EGTs were calculated using Eq. (3). The growth after temperature shifting was calculated using Eq. (4).

3.6. Analysis of estimation errors

Since the growth data were experimentally obtained and there were errors associated with each growth model representing a growth process, the errors may accumulate and propagate during the estimation of *C. perfringens* growth under fluctuating temperature conditions. The errors in the estimation of *C. perfringens* growth could be determined using a general error analysis equation (Dally et al., 1993), expressed as

$$\sigma_{L(t)} = \sqrt{\left(\frac{\partial L}{\partial A}\sigma_A\right)^2+\left(\frac{\partial L}{\partial B}\sigma_B\right)^2+\left(\frac{\partial L}{\partial \mu}\sigma_\mu\right)^2+\left(\frac{\partial L}{\partial M}\sigma_M\right)^2}, \quad (5)$$

where σ is the standard error.

The 95% confidence intervals of an estimate can be calculated by $L(t)\pm 1.96\sigma_{L(t)}$ (Ott, 1988). The absolute estimation errors, defined as the absolute value of the difference between the estimated value and the mean cell counts of a sampling point, were computed to represent the magnitude of the absolute errors.

4. Results and discussion

4.1. Isothermal growth curves

The average initial spore inoculum was 2.03 $\log_{10}$ (cfu/g) with a very small standard error (0.05). All isothermal growth curves were sigmoidal and could be fitted to the Gompertz model with relatively high degrees of accuracy (Fig. 2). For each Gompertz model, the lag phase (λ) and specific growth rate (K) could be determined using Eqs. (6) and (7) (Gibson et al., 1987), and the results are tabulated in Table 1. The mean asymptotic maximum cell population in the ground beef was 8.42 $\log_{10}$ (cfu/g) with a standard error of 0.01 $\log_{10}$ (cfu/g). This concentration was used as the maximum cell population in this study.

$$K = \frac{\mu(B-A)}{e}, \quad (6)$$

$$\lambda = M - \frac{1}{\mu}. \quad (7)$$

During the initial stage of bacterial growth, a slight decline in the bacterial concentration was frequently observed in the experiments. The initial decline in the cell counts during the lag phase of a growth curve may cause a computational problem when nonlinear regression is used to obtain a Gompertz equation. If the raw data containing an initial decline are used, the initial cell concentration (A) may be underestimated, which may lead to additional computational errors if the model is used to estimate the bacterial growth. Therefore, for practical purposes in this study, the initial decline was ignored and the data were adjusted to the initial inoculum level during the nonlinear regression.

4.2. Validation of the concept of equivalent growth time

To test the hypothesis of the EGT under different isothermal conditions, a series of experiments was conducted with a step change in the incubation temperature at different stages of bacterial growth. Fig. 3 depicts the effect of a step change in the incubation temperature on the growth of *C. perfringens* in cooked ground beef. In all these studies, samples were initially incubated at 30°C and then at 45°C. Figs. 3A–C represent growth studies with a step change in temperature at 2, 4 and 6 h after the first incubation periods. Since the lag phase duration of *C. perfringens* in cooked ground beef at 30°C was 3.67 h, changing the incubation temperature from 30°C to 45°C at 2, 4 and 6 h represented the temperature shifts within the lag phase, right after the completion of the lag phase, and within the exponential growth phase. In all of these figures, the growth of bacteria with a step change in temperature was estimated using the EGT concept.

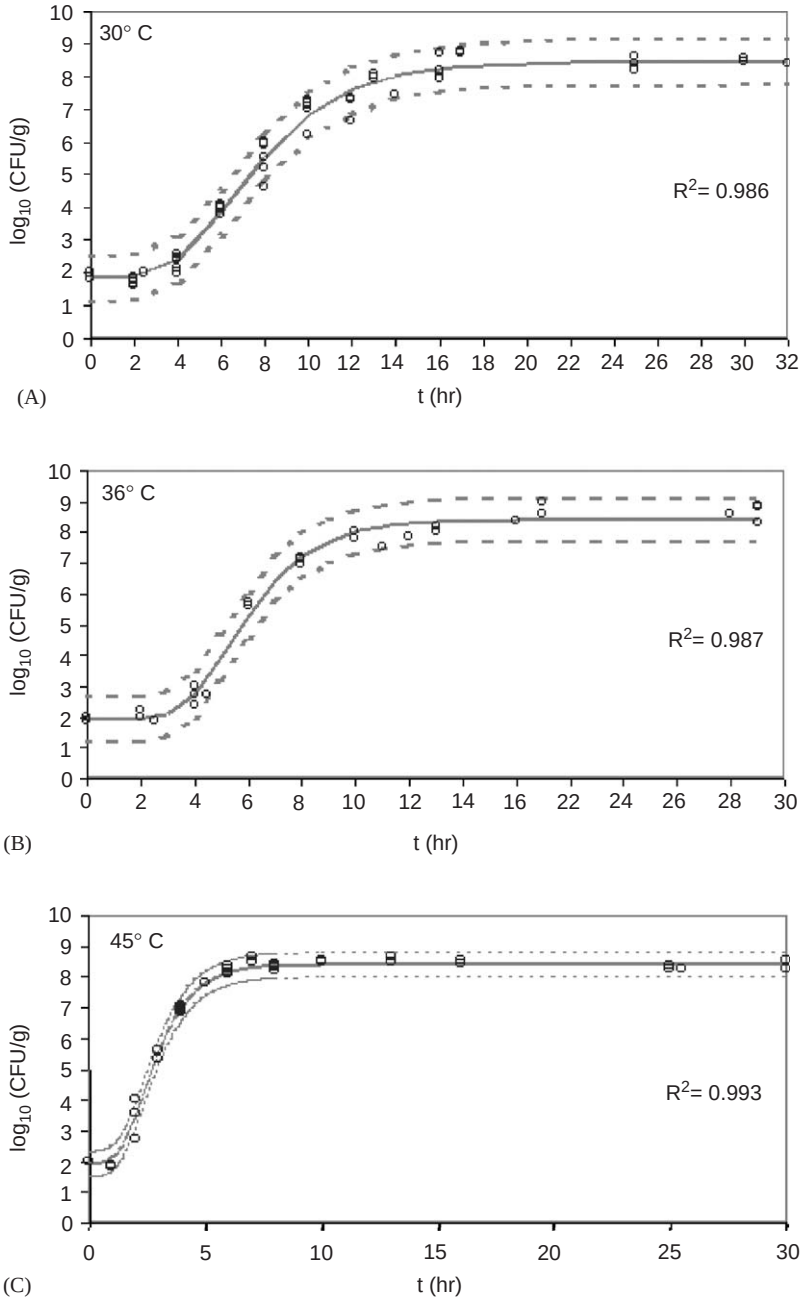

Fig. 2. The growth curves of *C. perfringens* in cooked beef at 30°C, 36°C and 45°C, respectively.

Table 1
Specific growth rates and lag phase durations of *C. perfringens* at 30°C, 36°C and 45°C

T (°C)	K (h^{-1})		M (h)		λ (h)	
	Mean	σ^a	Mean	σ^a	Mean	σ^a
30	0.868	0.048	6.474	0.161	3.671	0.278
36	1.379	0.108	5.257	0.136	3.529	0.224
45	2.118	0.071	2.400	0.039	1.272	0.065

[a] Standard error.

The absolute errors of estimation by this hypothesis were generally within $\pm 1 \log_{10}$ (cfu/g), except for a few data points in the experiments where the temperature was shifted at 2 h after the first incubation period (Fig. 4). These experiments represented the temperature shifts within the lag phase of the first incubation period. Larger estimation errors ($\approx 1.8 \log_{10}$ (cfu/g)) were observed within the first 2 h of the second incubation period. For *C. perfringens*, shifting the incubation temperatures within the lag phase may induce additional

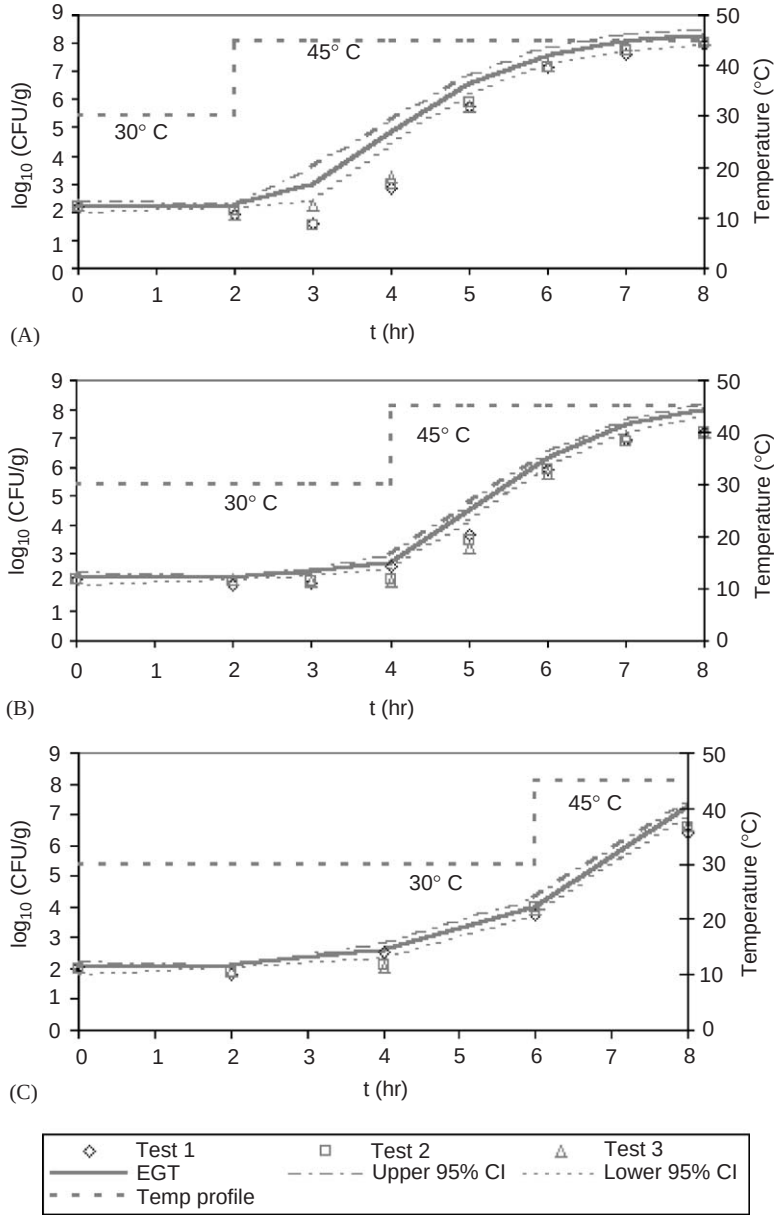

Fig. 3. Estimation of the growth of *C. perfringens* with a step change in temperature using the concept of the EGT.

stress for bacteria that apparently lead to a decline in the bacterial population. As a result, larger errors may be expected if the concept of EGT is used to estimate the growth of *C. perfringens* when a decline is significant in the initial period of growth.

4.3. Comparison with linear models

In predictive microbiology, a three-segment linear model (Eq. (8)) is frequently used to describe the isothermal bacterial growth. The three-segment linear model represents the lag, exponential, and stationary phases of an isothermal growth curve. The parameters, K and λ, in the linear models can be derived from the Gompertz equations (Buchanan et al., 1997)

$$L(t) = A, \quad t \leqslant \lambda$$
$$L(t) = A + K(t - \lambda), \quad \lambda < t < \frac{B - A}{\lambda} + \lambda$$
$$L(t) = B, \quad t > \lambda. \tag{8}$$

The estimation of bacterial growth must consider the physiological status of the bacteria if the linear model is used since the temperature fluctuations may occur at any point of the growth curves. For a temperature shift within the lag phase, Li and Torres (1993) proposed a methodology to estimate the completion of the lag phase under different temperature conditions. The basic

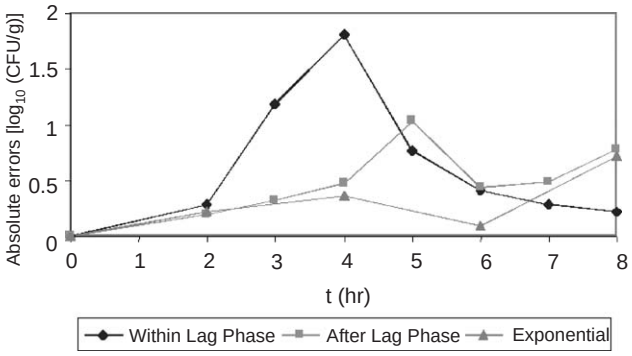

Fig. 4. The absolute errors of the estimation corresponding the growth curves in Fig. 3.

assumption of Li and Torres' method was based on the continuity of the bacterial growth. They postulated that there existed only one lag phase for the entire growth process regardless of the temperature conditions. Temperature fluctuations did not cause an increase in the lag phase. Under an isothermal condition, the incubation only contributed to a fraction of the lag phase if the incubation temperature was shifted before the completion of the lag phase (Eq. (9)). When the summation of all the lag phase fractions became a unity, then the lag phase was completed (Eq. (10)). According to Li and Torres (1993), the lag phase adaptation would start with $f_\lambda = 0$ and be completed when $F_\lambda = 1$:

$$f_{\lambda_i} = \frac{t_i}{\lambda_i}, \tag{9}$$

$$F_\lambda = \sum_{i=1}^{N} f_{\lambda_i}, \tag{10}$$

where t_i is the incubation time at temperature T_i, h; $f_{\lambda i}$ the lag phase fraction at temperature T_i; and F_λ the lag phase completion coefficient.

The traditional predictive microbiology must also consider the effect of temperature on the growth rate in the exponential phase. According to Baranyi et al. (1995), the specific growth rate is affected by temperature only, and the bacteria would instantaneously adapt to the new temperature environment as the incubation temperature changes. If the bacterial growth reaches the stationary phase, the cell concentration will become independent of temperature.

Based on the assumptions of Li and Torres (1993) and Baranyi et al. (1995), the estimation of bacterial growth under temperature fluctuations using the linear models is plotted and compared with the EGT concept in Fig. 5. For *C. perfringens* in ground beef, the linear models can accurately estimate the bacterial growth under fluctuating temperature conditions. However, the linear models would grossly overestimate the bacterial population at the transitional regions between the exponential and stationary phases.

4.4. Comparison with Zwietering's method

Zwietering et al. (1994) also proposed a methodology to estimate the bacterial growth with a step change in temperature using a modified Gompertz model. The authors hypothesized that the temperature shift would lead to a 25% increase in the lag phase. If temperature shifting occurs within the lag phase of the first incubation period, the lag phase has to be completed when the bacteria are incubated under the second incubation temperature. According to Zwietering et al. (1994), the lag phase of the second incubation period, λ_{shift}, can be calculated from Eq. (11) and the bacterial growth after temperature shifting calculated from a modified Gompertz equation (Eq. (12)).

$$\lambda_{shift} = (1.25 - f_{\lambda_1})\lambda_2 \tag{11}$$

$$L(t) = A + (B - A) \times \exp\{-\exp[-\mu_2(\lambda_{shift} - \langle t - t_s \rangle) - \mu]\} \tag{12}$$

where μ_2 is the relative growth rate at the second incubation temperature, h^{-1}; t_s is the time at which the temperature is shifted, h.

If the step change in temperature occurs within the exponential phase, Zwietering et al. (1994) postulated that the temperature shifting would induce an additional lag time, λ_{shift}, which is equal to 25% of the lag phase duration (λ_2) under the second isothermal condition. The growth of bacteria after temperature shifting can be estimated from

$$L(t) = L_s(t) + [(B - A) - L_s(t)] \times \exp\{-\exp[\mu_2(\lambda_{shift} - \langle t - t_s \rangle) - \mu]\}. \tag{13}$$

Although more sophisticated in theory, the accuracy of the Zwietering's method was very close to the methodology proposed in this study (Fig. 5). Even considering the increased lag time caused by temperature shifting, the estimation accuracy was not significantly improved in the cases where the temperature was shifted within the lag phase (Fig 5A). In the conclusions drawn by the authors, according to Zwietering et al. (1994), the additional lag time could be neglected in the estimation of bacterial growth.

4.5. Application of the EGT concept

The concept of EGT was tested under multiple fluctuating temperature conditions (Figs. 6 and 7). Shown in Fig. 6 is the estimation of the bacterial growth with temperatures fluctuating between 30°C and 45°C, starting at a lower temperature (30°C). In these experiments the first temperature shift occurred before the completion of the lag phase at 30°C. Fig. 7 illustrates the estimation of bacterial growth with the temperatures fluctuating between 45°C and 36°C, starting at 45°C.

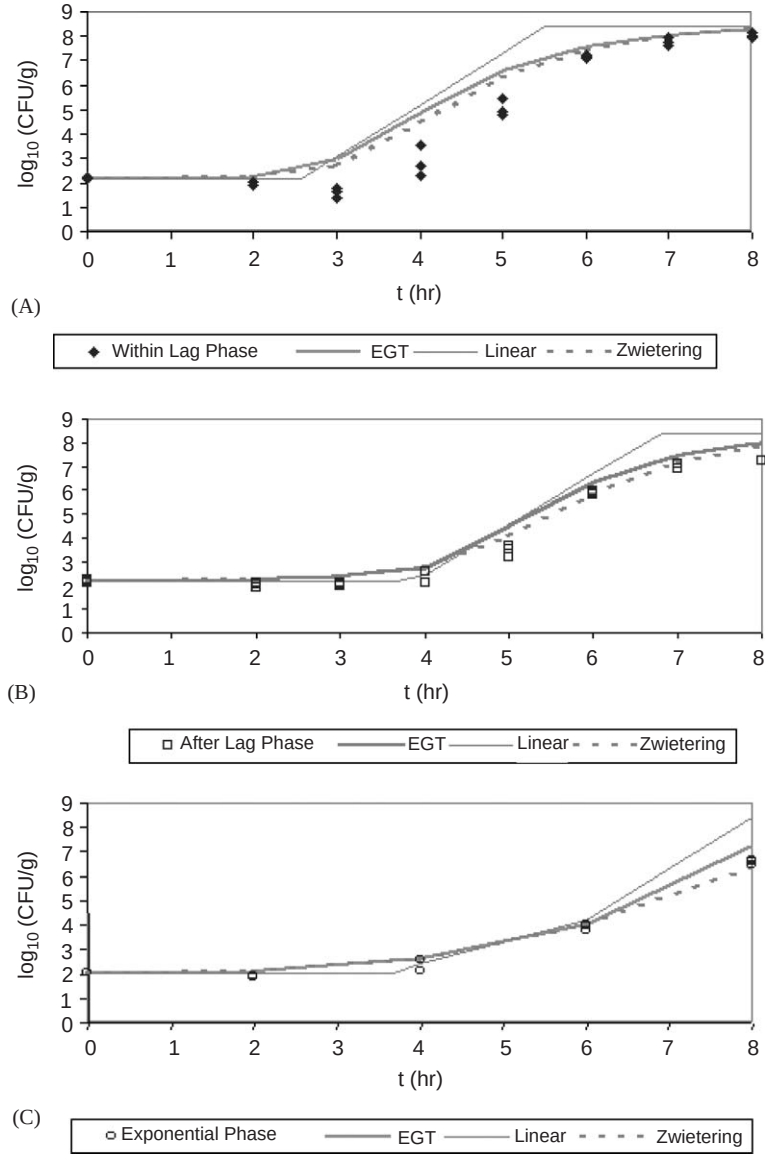

Fig. 5. Comparison of different methods of estimation.

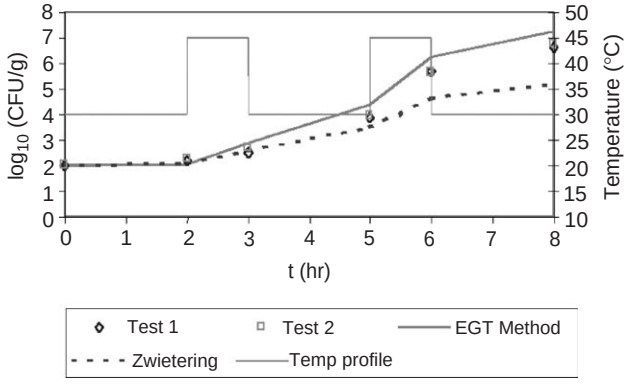

Fig. 6. Estimation of the growth of *C. perfringens* at temperatures fluctuating between 30°C and 45°C.

In these tests, however, the lag phase was almost completed (98.3%) when the first temperature shift occurred. Figs. 6 and 7 demonstrate that the EGT method could accurately estimate the bacterial growth with multiple temperature fluctuations. As a comparison, the estimation of bacterial growth using the Zwietering's method was also plotted in Figs. 6 and 7. Clearly shown in these figures, the methodology proposed by Zwietering et al. (1994) failed to provide accurate estimations of the bacterial growth under multiple fluctuating temperature conditions. The imposed additional lag times at each step of the temperature change attributed to the accumulation of the errors and the failure of the method.

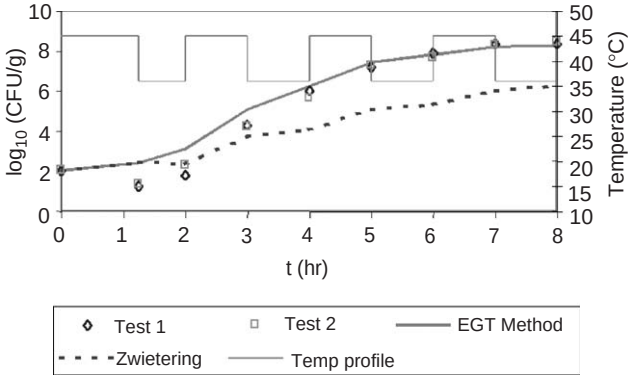

Fig. 7. Estimation of the growth of C. perfringens at temperatures fluctuating between 45°C and 36°C.

5. Conclusions

The concept of equivalent growth time (EGT), proposed in this study was tested successfully in estimating the bacterial growth under fluctuating temperature conditions. Using the EGT concept the accuracy of estimation of the growth of *C. perfringens* in cooked ground beef under temperature fluctuations can generally be within $\pm 1.0 \log_{10}$ (cfu/g). Although the development of the concept of EGT was based on the growth studies of *C. perfringens* spores in cooked ground beef, this methodology can be potentially extended to other foodborne pathogens.

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